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(54) Polymorphisms in the human P2X7 gene

(57) This invention relates to polymorphisms in the human $P2X_7$ gene and corresponding novel allelic polypeptides encoded thereby. The invention also relates to methods and materials for analysing allelic variation in the $P2X_7$ gene, and to the use of $P2X_7$ polymorphism in treatment of diseases with $P2X_7$ drugs.

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Description

[0001] This invention relates to polymorphisms in the human $P2X_7$ gene and corresponding novel allelic polypeptides encoded thereby. The invention also relates to methods and materials for analysing allelic variation in the $P2X_7$ gene, and to the use of $P2X_7$ polymorphism in treatment of diseases with $P2X_7$ drugs.

[0002] The $P2X_7$ receptor (previously known as P2Z receptor), which is a ligand-gated ion channel, is present on a variety of cell types, largely those known to be involved in the inflammatory/immune process, specifically, macrophages, mast cells and lymphocytes (T and B). Activation of the $P2X_7$ receptor by extracellular nucleotides, in particular adenosine triphosphate, leads to the release of interleukin-1β (IL-1β) and giant cell formation (macrophages/microglial cells), degranulation (mast cells) and L-selectin shedding (lymphocytes). $P2X_7$ receptors are also located on antigen-presenting cells (APC), keratinocytes, salivary acinar cells (parotid cells) and hepatocytes. Compounds acting at the $P2X_7$ receptor are therefore indicated as pharmaceuticals for use in the treatment of rheumatoid arthritis, osteoarthritis, psoriasis, allergic dermatitis, asthma, chronic obstructive pulmonary disease (COPD), hyperresponsiveness of the airway, septic shock, glomerulonephritis, irritable bowel disease, Crohn's disease, ulcerative colitis, atherosclerosis, growth and metastases of malignant cells, myoblastic leukaemia, diabetes, Alzheimer's disease, meningitis, osteoporosis, burn injury, ischaemic heart disease, stroke and varicose veins. For further background, the reader is referred to the following articles: North and Barnard in Current Opinion in Neurobiology 1997, **7**, 346-357: Rassendren, JBC, 1997, **273**, 5482-6; and Buell, Receptors and Channels, 1998, **5**, 347-354. The terms $P2X_7$, $P2X_7$ receptor and P2RX7 are used interchangeably herein.

[0003] All positions herein of polymorphisms in the 5' UTR region of the $P2X_7$ polynucleotide relate to the position in SEQ ID NO 1 unless stated otherwise or apparent from the context.

[0004] All positions herein of polymorphisms in the exon regions of the $P2X_7$ polynucleotide relate to the position in SEQ ID NO 2 unless stated otherwise or apparent from the context.

[0005] All positions herein of polymorphisms in the intron regions of the $P2X_7$ polynucleotide relate to the position in SEQ ID NO 3 unless stated otherwise or apparent from the context.

[0006] All positions herein of polymorphisms in the $P2X_7$ polypeptide relate to the position in SEQ ID NO 4 unless stated otherwise or apparent from the context.

[0007] One approach is to use knowledge of polymorphisms to help identify patients most suited to therapy with particular pharmaceutical agents (this is often termed "pharmacogenetics"). Pharmacogenetics can also be used in pharmaceutical research to assist the drug selection process. Polymorphisms are used in mapping the human genome and to elucidate the genetic component of diseases. The reader is directed to the following references for background details on pharmacogenetics and other uses of polymorphism detection: Linder *et al.* (1997), Clinical Chemistry, **43**, 254; Marshall (1997), Nature Biotechnology, **15**, 1249; International Patent Application WO 97/40462, Spectra Biomedical; and Schafer *et al.* (1998), Nature Biotechnology, **16**, 33.

[0008] Clinical trials have shown that patient response to treatment with pharmaceuticals is often heterogeneous. Thus there is a need for improved approaches to pharmaceutical agent design and therapy.

[0009] Point mutations in polypeptides will be referred to as follows: natural amino acid (using 1 or 3 letter nomenclature) , position, new amino acid. For (a hypothetical) example "D25K" or "Asp25Lys" means that at position 25 an aspartic acid (D) has been changed to lysine (K). Multiple mutations in one polypeptide will be shown between square brackets with individual mutations separated by commas.

[0010] The present invention is based on the discovery of polymorphisms in $P2X_7$. In particular, we have found thirty polymorphisms in the coding sequence of the $P2X_7$ gene, 12 of which lead to changes in the sequence of expressed protein.

[0011] According to one aspect of the present invention there is provided a method for the diagnosis of a polymorphism in $P2X_7$ in a human, which method comprises determining the sequence of the human at at least one polymorphic position and determining the status of the human by reference to polymorphism in $P2X_7$. Preferred polymorphic positions are one or more of the following positions:

positions 936, 1012, 1147, 1343 and 1476 in the 5'UTR region of the $P2X_7$ gene as defined by the position in SEQ ID NO: 1;
positions 253, 488, 489, 760, 835, 853, 1068, 1096, 1315, 1324, 1405, 1448, 1494, 1513, 1628 and 1772 in the coding region of the $P2X_7$ gene as defined by the position in SEQ ID NO: 2; and
positions 4780, 4845, 4849, 5021, 5554, 5579, 5535, 5845 and 6911 in the intron region of the $P2X_7$ gene as defined by the position in SEQ ID NO: 3;
positions 76 ,155, 245, 270, 276, 348, 357, 430, 433, 460, 490 and 496 in the P2X7 polypeptide as defined by the position in SEQ ID NO: 4.

[0012] The term human includes both a human having or suspected of having a $P2X_7$ mediated disease and an

asymptomatic human who may be tested for predisposition or susceptibility to such disease. At each position the human may be homozygous for an allele or the human may be a heterozygote.

[0013] The term "status" refers to the genetic status of the human as detected by potential sequence variation at defined positions of a polynucleotide or corresponding protein. The term "diagnosis of a polymorphism" refers to determination of the genetic status of an individual at a polymorphic position (in which the indiviual may be homozygous or heterozygous at each position).

[0014] The term polymorphism includes single nucleotide substitution, nucleotide insertion and nucleotide deletion which in the case of insertion and deletion includes insertion or deletion of one or more nucleotides at a position of a gene and corresponding alterations in expressed protein.

[0015] In one embodiment of the invention preferably the method for diagnosis described herein is one in which the polymorphism in the in the 5'UTR region of the $P2X_7$ gene as defined by the position in SEQ ID NO: 1 is any one of the following:

at position 936 is presence of C and/or A; at position 1012 is presence of T and/or C;
at position 1147 is presence of A and/or G; at position 1343 is presence of G and/or A; and
at position 1476 is presence of A and/or G.

[0016] In one embodiment of the invention preferably the method for diagnosis described herein is one in which the polymorphism in the coding region of the $P2X_7$ gene as defined by the position in SEQ ID NO: 2 is any one of the following:

at position 253 is presence of T and/or C; at position 488 is presence of G and/or A;
at position 489 is presence of C and/or T; at position 760 is presence of T and/or G;
at position 835 is presence of G and/or A; at position 853 is presence of G and/or A;
at position 1068 is presence of G and/or A; at position 1096 is presence of C and/or G;
at position 1315 is presence of C and/or G; at position 1324 is presence of C and/or T;
at position 1405 is presence of A and/or G; at position 1448 is presence of C and/or T;
at position 1494 is presence of A and/or G; at position 1513 is presence of A and/or C;
at position 1628 is presence of G and/or T; and at position 1772 is presence of G and/or A.

[0017] In one embodiment of the invention preferably the method for diagnosis described herein is one in which the polymorphism in the intron region of the $P2X_7$ gene as defined by the position in SEQ ID NO: 3. is any one of the following:

at position 4780 is presence of C and/or T; at position 4845 is presence of C and/or T;
at position 4849 is presence of A and/or C; at position 5021 is presence of T and/or C;
at position 5554 is presence of 3 and/or 4 repeats of GTTT (wherein position 5554 refers to the position of the G in the first unit repeat):
at position 5579 is presence of G and/or C; at position 5535 is presence of A and/or T;
at position 5845 is presence of C and/or T; and at position 6911 is presence of T and/or C.

[0018] In one embodiment of the invention preferably the method for diagnosis described herein is one in which the polymorphism in the $P2X_7$ protein as defined by the position in SEQ ID NO: 4. is any one of the following: val76ala, his155tyr, val245gly. arg270his, arg276his, ala348thr, thr357ser, pro430arg, ala433val, gln460arg, ser490gly and glu496ala.

[0019] The method for diagnosis is preferably one in which the sequence is determined by a method selected from amplification refractory mutation system, restriction fragment length polymorphism and primer extension.

[0020] The status of the individual may be determined by reference to allelic variation at any 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30 or more positions.

[0021] The test sample of nucleic acid is conveniently a sample of blood, bronchoalveolar lavage fluid, sputum, or other body fluid or tissue obtained from an individual. It will be appreciated that the test sample may equally be a nucleic acid sequence corresponding to the sequence in the test sample, that is to say that all or a part of the region in the sample nucleic acid may firstly be amplified using any convenient technique e.g. PCR, before analysis of allelic variation.

[0022] It will be apparent to the person skilled in the art that there are a large number of analytical procedures which may be used to detect the presence or absence of variant nucleotides at one or more polymorphic positions of the invention. In general, the detection of allelic variation requires a mutation discrimination technique, optionally an amplification reaction and optionally a signal generation system. Table 1 lists a number of mutation detection techniques,

some based on the PCR. These may be used in combination with a number of signal generation systems, a selection of which is listed in Table 2. Further amplification techniques are listed in Table 3. Many current methods for the detection of allelic variation are reviewed by Nollau *et al.*. Clin. Chem. **43**, 1114-1120. 1997; and in standard textbooks. for example "Laboratory Protocols for Mutation Detection", Ed. by U. Landegren. Oxford University Press, 1996 and "PCR", 2nd Edition by Newton & Graham. BIOS Scientific Publishers Limited, 1997.

Abbreviations:

[0023]

ALEX™	Amplification refractory mutation system linear extension
APEX	Arrayed primer extension
ARMS™	Amplification refractory mutation system
b-DNA	Branched DNA
bp	base pair
CMC	Chemical mismatch cleavage
COPS	Competitive oligonucleotide priming system
DGGE	Denaturing gradient gel electrophoresis
ELISA	Enzyme Linked Immuno Sorbent Assay
FRET	Fluorescence resonance energy transfer
LCR	Ligase chain reaction
MASDA	Multiple allele specific diagnostic assay
NASBA	Nucleic acid sequence based amplification
OLA	Oligonucleotide ligation assay
PCR	Polymerase chain reaction
PTT	Protein truncation test
RFLP	Restriction fragment length polymorphism
SDA	Strand displacement amplification
SNP	Single nucleotide polymorphism
SSCP	Single-strand conformation polymorphism analysis
SSR	Self sustained replication
TGGE	Temperature gradient gel electrophoresis

Table 1 -

Mutation Detection Techniques

General: DNA sequencing, Sequencing by hybridisation

Scanning: PTT*, SSCP, DGGE, TGGE, Cleavase, Heteroduplex analysis, CMC, Enzymatic mismatch cleavage

Hybridisation Based

Solid phase hybridisation: Dot blots, MASDA, Reverse dot blots, Oligonucleotide arrays (DNA Chips).

Solution phase hybridisation: Taqman™ - US-5210015 & US-5487972 (Hoffmann-La Roche), Molecular Beacons - Tyagi *et al* (1996), Nature Biotechnology, **14**, 303; WO 95/13399 (Public Health Inst., New York)

Extension Based: ARMS™, ALEX™ - European Patent No. EP 332435 B1 (Zeneca Limited), COPS - Gibbs *et al* (1989), Nucleic Acids Research, **17**, 2347.

Incorporation Based: Mini-sequencing, APEX

Restriction Enzyme Based: RFLP, Restriction site generating PCR

* Note: not useful for detection of promoter polymorphisms.

Table 1 - (continued)

Mutation Detection Techniques
Ligation Based: OLA
Other: Invader assay

Table 2 -

Signal Generation or Detection Systems
Fluorescence: FRET, Fluorescence quenching, Fluorescence polarisation - United Kingdom Patent No. 2228998 (Zeneca Limited)
Other: Chemiluminescence, Electrochemiluminescence, Raman, Radioactivity, Colorimetric, Hybridisation protection assay, Mass spectrometry

Table 3 -
Further Amplification Methods
SSR, NASBA, LCR, SDA, b-DNA

Table 4-
Protein variation detection methods
Immunoassay
Immunohistology
Peptide sequencing

[0024] Preferred mutation detection techniques include ARMS™, ALEX™, COPS, Taqman, Molecular Beacons, RFLP, and restriction site based PCR and FRET techniques. Immunoassay techniques are known in the art e.g. A Practical Guide to ELISA by D M Kemeny, Pergamon Press 1991; Principles and Practice of Immunoassay, 2nd edition, C P Price & D J Newman, 1997, published by Stockton Press in USA & Canada and by Macmillan Reference in the United Kingdom. Histological techniques are described in Theory and Practice of Histological Techniques by J D Bancroft & A Stevens, 4th Edition, Churchill Livingstone,1996. Protein sequencing is described in Laboratory Techniques in Biochemistry and Molecular Biology, Volume 9, Sequencing of Proteins and Peptides, G Allen, 2nd revised edition, Elsevier, 1989. Particularly preferred methods include ARMS™ and RFLP based methods. ARMS™ is an especially preferred method.

[0025] In a further aspect, the diagnostic methods of the invention are used to assess the pharmacogenetics of a drug acting at $P2X_7$.

[0026] Assays, for example reporter-based assays, may be devised to detect whether one or more of the above polymorphisms affect transcription levels and/or message stability.

[0027] Individuals who carry particular allelic variants of the $P2X_7$ gene may therefore exhibit differences in their ability to regulate protein biosynthesis under different physiological conditions and will display altered abilities to react to different diseases. In addition, differences arising as a result of allelic variation may have a direct effect on the response of an individual to drug therapy. The diagnostic methods of the invention may be useful both to predict the clinical response to such agents and to determine therapeutic dose.

[0028] In a further aspect, the diagnostic methods of the invention, are used to assess the predisposition and/or susceptibility of an individual to diseases mediated by $P2X_7$. This may be particularly relevant in the development of hyperlipoproteinemia and cardiovascular disease and the present invention may be used to recognise individuals who are particularly at risk from developing these conditions.

[0029] In a further aspect, the diagnostic methods of the invention are used in the development of new drug therapies which selectively target one or more allelic variants of the $P2X_7$ gene. Identification of a link between a particular allelic variant and predisposition to disease development or response to drug therapy may have a significant impact on the design of new drugs. Drugs may be designed to regulate the biological activity of variants implicated in the disease process whilst minimising effects on other variants.

[0030] In a further diagnostic aspect of the invention the presence or absence of variant nucleotides is detected by reference to the loss or gain of, optionally engineered, sites recognised by restriction enzymes.

[0031] According to another aspect of the present invention there is provided a human $P2X_7$ gene or its complementary strand comprising a variant allelic polymorphism at one or more of positions defined herein or a fragment thereof

of at least 20 bases comprising at least one novel polymorphism.

[0032] Fragments are at least 17 bases, more preferably at least 20 bases, more preferably at least 30 bases.

[0033] According to another aspect of the present invention there is provided a polynucleotide comprising at least 20 bases of the human $P2X_7$ gene and comprising a polymorphism selected from any one of the following:

Region	Polymorphism SEQ ID NO: 1
5'UTR	936 C→A 1012 T→C 1147 A→G 1343 G→A 1476 A→G

Region	Polymorphism SEQ ID NO: 2
exon 2	253 T→C
exon 5	488 G→A 489 C→T
exon 7	760 T→G
exon 8	835 G→A 853 G→A
exon 11	1068 G→A 1096 C→G
exon 12	1315 C→G
exon 13	1324 C→T 1405 A→G 1448 C→T 1494 A→G 1513 A→C 1628 G→T 1772 G→A

Region	Polymorphism SEQ ID NO: 3
intron E	4780 C→T 4845 C→T 4849 A→C
intron F	5021 T→C 5554 (GTTT)n=3,4 5579 G→C 5535 A→T
intron G	5845 C→T 6911 T→C

[0034] According to another aspect of the present invention there is provided a polynucleotide comprising at least 20 bases of the human $P2X_7$ gene and comprising an allelic variant selected from any one of the following:

Region	Variant SEQ ID NO: 1
5'UTR	936 A 1012 C

(continued)

Region	Variant SEQ ID NO: 1
	1147 G 1343 A 1476 G

Region	Variant SEQ ID NO: 2
exon 2	253 C
exon 5	488 A 489 T
exon 7	760 G
exon 8	835 A 853 A
exon 11	1068 A 1096 G
exon 12	1315 G
exon 13	1324 T 1405 G 1448 T 1494 G 1513 C 1628 T 1772 A

Region	Variant SEQ ID NO: 3
intron E	4780 T 4845 T 4849 C
intron F	5021 C 5554 $(GTTT)_n$,n=4 5579 C 5535 T
intron G	5845 T 6911 C

[0035] According to another aspect of the present invention there is provided a human $P2X_7$ gene or its complementary strand comprising a polymorphism, preferably corresponding with one or more the positions defined herein or a fragment thereof of at least 20 bases comprising at least one polymorphism.

[0036] Fragments are at least 17 bases, more preferably at least 20 bases, more preferably at least 30 bases.

[0037] The invention further provides a nucleotide primer which can detect a polymorphism of the invention.

[0038] According to another aspect of the present invention there is provided an allele specific primer capable of detecting a $P2X_7$ gene polymorphism, preferably at one or more of the positions as defined herein.

[0039] An allele specific primer is used, generally together with a constant primer, in an amplification reaction such as a PCR reaction, which provides the discrimination between alleles through selective amplification of one allele at a particular sequence position e.g. as used for **ARMS™** assays. The allele specific primer is preferably 17- 50 nucleotides, more preferably about 17-35 nucleotides, more preferably about 17-30 nucleotides.

[0040] An allele specific primer preferably corresponds exactly with the allele to be detected but derivatives thereof

are also contemplated wherein about 6-8 of the nucleotides at the 3' terminus correspond with the allele to be detected and wherein up to 10, such as up to 8, 6, 4, 2, or 1 of the remaining nucleotides may be varied without significantly affecting the properties of the primer.

[0041] Primers may be manufactured using any convenient method of synthesis. Examples of such methods may be found in standard textbooks, for example "Protocols for Oligonucleotides and Analogues; Synthesis and Properties," Methods in Molecular Biology Series; Volume 20; Ed. Sudhir Agrawal, Humana ISBN: 0-89603-247-7; 1993; 1st Edition. If required the primer(s) may be labelled to facilitate detection.

[0042] According to another aspect of the present invention there is provided an allele-specific oligonucleotide probe capable of detecting a $P2X_7$ gene polymorphism, preferably at one or more of the positions defined herein.

[0043] The allele-specific oligonucleotide probe is preferably 17- 50 nucleotides, more preferably about 17-35 nucleotides, more preferably about 17-30 nucleotides.

[0044] The design of such probes will be apparent to the molecular biologist of ordinary skill. Such probes are of any convenient length such as up to 50 bases, up to 40 bases, more conveniently up to 30 bases in length, such as for example 8-25 or 8-15 bases in length. In general such probes will comprise base sequences entirely complementary to the corresponding wild type or variant locus in the gene. However, if required one or more mismatches may be introduced, provided that the discriminatory power of the oligonucleotide probe is not unduly affected. The probes of the invention may carry one or more labels to facilitate detection.

[0045] According to another aspect of the present invention there is provided an allele specific primer or an allele specific oligonucleotide probe capable of detecting a $P2X_7$ gene polymorphism at one of the positions defined herein.

[0046] According to another aspect of the present invention there is provided a diagnostic kit comprising an allele specific oligonucleotide probe of the invention and/or an allele-specific primer of the invention.

[0047] The diagnostic kits may comprise appropriate packaging and instructions for use in the methods of the invention. Such kits may further comprise appropriate buffer(s) and polymerase(s) such as thermostable polymerases, for example taq polymerase.

[0048] In another aspect of the invention, the polymorphisms of this invention may be used as genetic markers in linkage studies. This particularly applies to the polymorphisms of relatively high frequency. The $P2X_7$ gene is on chromosome 12q24 (Buell et al, Receptors and Channels, 1998, 5,347-354). Low frequency polymorphisms may be particularly useful for haplotyping as described below. A haplotype is a set of alleles found at linked polymorphic sites (such as within a gene) on a single (paternal or maternal) chromosome. If recombination within the gene is random, there may be as many as 2^n haplotypes, where 2 is the number of alleles at each SNP and n is the number of SNPs. One approach to identifying mutations or polymorphisms which are correlated with clinical response is to carry out an association study using all the haplotypes that can be identified in the population of interest. The frequency of each haplotype is limited by the frequency of its rarest allele, so that SNPs with low frequency alleles are particularly useful as markers of low frequency haplotypes. As particular mutations or polymorphisms associated with certain clinical features, such as adverse or abnormal events, are likely to be of low frequency within the population, low frequency SNPs may be particularly useful in identifying these mutations (for examples see: Linkage disequilibrium at the cystathionine beta synthase (CBS) locus and the association between genetic variation at the CBS locus and plasma levels of homocysteine. *Ann Hum Genet* (1998) **62**:481-90, De Stefano V, Dekou V, Nicaud V, Chasse JF, London J, Stansbie D, Humphries SE, and Gudnason V; and Variation at the von willebrand factor (vWF) gene locus is associated with plasma vWF:Ag levels: identification of three novel single nucleotide polymorphisms in the vWF gene promoter. *Blood* (1999) **93**:4277-83, Keightley AM, Lam YM, Brady JN, Cameron CL, Lillicrap D).

[0049] According to another aspect of the present invention there is provided a computer readable medium comprising at least one novel sequence of the invention stored on the medium. The computer readable medium may be used, for example, in homology searching, mapping, haplotyping, genotyping or pharmacogenetic analysis.

[0050] According to another aspect of the present invention there is provided a method of treating a human in need of treatment with a drug acting at $P2X_7$ in which the method comprises:

i) diagnosis of a polymorphism in $P2X_7$ in the human, which diagnosis preferably comprises determining the sequence at one or more of the following positions:

[0051]

positions 936, 1012, 1147, 1343 and 1476 in the 5'UTR region of the $P2X_7$ gene as defined by the position in SEQ ID NO: 1;

positions 253, 488, 489, 760, 835, 853, 1068, 1096, 1315, 1324, 1405, 1448, 1494, 1513, 1628 and 1772 in the coding region of the $P2X_7$ gene as defined by the position in SEQ ID NO: 2; and

positions 4780, 4845, 4849, 5021, 5554, 5579, 5535, 5845 and 6911 in the intron region of the $P2X_7$ gene as defined by the position in SEQ ID NO: 3; and

positions 76 ,155, 245, 270, 276, 348, 357, 430, 433, 460, 490 and 496 in the $P2X_7$ polypeptide as defined by the position in SEQ ID NO: 4;
and determining the status of the human by reference to polymorphism in $P2X_7$; and

ii) administering an effective amount of the drug.

[0052] Preferably determination of the status of the human is clinically useful. Examples of clinical usefulness include deciding which drug or drugs to administer and/or in deciding on the effective amount of the drug or drugs. The term "drug acting at P2X7" means that drug binding with P2X7 in humans is an important part of a drug exerting its pharmaceutical effect in man. Compounds which are known to be antagonists of the P2X7 receptor are described in published PCT application nos. WO 99/29660, WO 99/29661, WO 99/29686, WO 00/61569, WO 00/71529, WO 01/42194, WO 01/44170, WO 01/44213 and WO 01/46200. According to another aspect of the present invention there is provided use of a drug acting at $P2X_7$ in preparation of a medicament for treating a disease in a human diagnosed as having a polymorphism therein, preferably at one or more of the positions defined herein.

[0053] According to another aspect of the present invention there is provided a pharmaceutical pack comprising $P2X_7$ drug and instructions for administration of the drug to humans diagnostically tested for a polymorphism therein, preferably at one or more of the positions defined herein.

[0054] According to another aspect of the present invention there is provided an allelic variant of human $P2X_7$ polypeptide comprising at least one of the following:

a alanine at position 76 of SEQ ID NO 4;
a tyrosine at position 155 of SEQ ID NO 4;
a glycine at position 245 of SEQ ID NO 4;
a histidine at position 270 of SEQ ID NO 4;
a histidine at position 276 of SEQ ID NO 4;
a threonine at position 348 of SEQ ID NO 4;
a serine at position 357 of SEQ ID NO 4;
a arginine at position 430 of SEQ ID NO 4;
a valine at position 433 of SEQ ID NO 4;
a arginine at position 460 of SEQ ID NO 4;
a glycine at position 490 of SEQ ID NO 4; and
a glutamic acid at position 496 of SEQ ID NO 4;

or a fragment thereof comprising at least 10 amino acids provided that the fragment comprises at least one allelic variant.

[0055] Fragments of polypeptide are at least 10 amino acids, more preferably at least 15 amino acids, more preferably at least 20 amino acids.

[0056] According to another aspect of the present invention there is provided an antibody specific for an allelic variant of human $P2X_7$ polypeptide as described herein.

[0057] Antibodies can be prepared using any suitable method. For example, purified polypeptide may be utilized to prepare specific antibodies. The term "antibodies" is meant to include polyclonal antibodies, monoclonal antibodies, and the various types of antibody constructs such as for example $F(ab')_2$, Fab and single chain Fv. Antibodies are defined to be specifically binding if they bind the allelic variant of $P2X_7$ with a K_a of greater than or equal to about 10^7 M^{-1}. Affinity of binding can be determined using conventional techniques, for example those described by Scatchard et al., *Ann. N.Y. Acad. Sci.*, 51:660 (1949).

[0058] Polyclonal antibodies can be readily generated from a variety of sources, for example, horses, cows, goats, sheep, dogs, chickens, rabbits, mice or rats, using procedures that are well-known in the art. In general, antigen is administered to the host animal typically through parenteral injection. The immunogenicity of antigen may be enhanced through the use of an adjuvant, for example, Freund's complete or incomplete adjuvant. Following booster immunizations, small samples of serum are collected and tested for reactivity to antigen. Examples of various assays useful for such determination include those described in: *Antibodies: A Laboratory Manual,* Harlow and Lane (eds.), Cold Spring Harbor Laboratory Press, 1988; as well as procedures such as countercurrent immuno-electrophoresis (CIEP), radioimmunoassay, radioimmunoprecipitation, enzyme-linked immuno-sorbent assays (ELISA), dot blot assays, and sandwich assays, see U.S. Patent Nos. 4,376,110 and 4,486,530.

[0059] Monoclonal antibodies may be readily prepared using well-known procedures, see for example, the procedures described in U.S. Patent Nos. RE 32,011, 4,902,614, 4,543,439 and 4,411,993; Monoclonal Antibodies, Hybridomas: *A New Dimension in Biological Analyses,* Plenum Press, Kennett, McKearn, and Bechtol (eds.), (1980).

[0060] The monoclonal antibodies of the invention can be produced using alternative techniques, such as those

described by Alting-Mees et al., "Monoclonal Antibody Expression Libraries: A Rapid Alternative to Hybridomas", *Strategies in Molecular Biology* 3: 1-9 (1990) which is incorporated herein by reference. Similarly, binding partners can be constructed using recombinant DNA techniques to incorporate the variable regions of a gene that encodes a specific binding antibody. Such a technique is described in Larrick et aL, *Biotechnology*, 7: 394 (1989).

[0061] Once isolated and purified, the antibodies may be used to detect the presence of antigen in a sample using established assay protocols, see for example "A Practical Guide to EUSA" by D. M. Kemeny, Pergamon Press, Oxford, England.

[0062] According to another aspect of the invention there is provided a diagnostic kit comprising an antibody of the invention.

[0063] According to another aspect of the present invention there is provided a polynucleotide comprising any one of the following twenty six $P2X_7$ haplotypes:

	1012	489	5579	835	853	1068	1096	1405	1513
	SEQ ID 1	SEQ ID 2	SEQ ID 3	SEQ ID 2	SEQ ID 2	SEQ ID 2	SEQ ID 2	SEQ ID 2	SEQ ID 2
1	T	T	C	G	G	A	G	A	A
2	C	C	G	G	G	G	C	A	A
3	C	C	C	A	G	G	C	A	C
4	C	T	G	G	G	A	C	G	A
5	C	C	G	G	G	A	G	A	A
6	C	C	C	A	G	G	C	A	A
7	T	T	G	G	G	A	C	G	A
8	C	T	C	G	G	G	C	A	A
9	C	C	C	G	G	A	C	A	A
10	C	T	G	G	G	G	C	A	C
11	T	C	G	G	G	A	C	A	A
12	C	T	C	G	G	G	C	A	C
13	T	C	C	G	G	A	C	A	A
14	T	C	C	G	G	G	C	A	C
15	C	T	C	G	G	A	C	A	A
16	T	T	C	G	G	A	C	G	A
17	C	C	G	G	G	A	C	G	A
18	T	C	G	A	A	G	C	A	A
19	C	C	C	G	G	G	G	A	A
20	T	C	C	G	G	G	G	A	A
21	C	T	C	A	G	G	C	A	A
22	C	C	C	G	G	G	C	A	C
23	C	T	G	G	A	A	G	G	A
24	T	T	G	G	G	A	G	G	A
25	C	T	C	G	G	G	G	A	A
26	C	C	C	G	G	G	C	A	A

[0064] According to another aspect of the present invention there is provided a human $P2X_7$ polypeptide comprising one of the following eighteen combinations of alleleic variant determined amino acids based on positions identified in SEQ ID NO: 4:

	155	270	276	348	357	460	496
1	Y	R	R	T	S	Q	E
2	Y	R	R	T	T	R	E
3	Y	R	R	T	T	Q	E
4	Y	R	R	T	S	R	E
5	Y	R	R	A	T	Q	A
6	Y	R	R	A	T	Q	E
7	Y	R	R	A	S	Q	E
8	Y	R	H	T	S	R	E
9	Y	H	R	A	T	Q	E
10	H	R	R	T	T	Q	E
11	H	R	R	T	T	R	E
12	H	R	R	A	T	Q	A
13	H	R	R	A	S	Q	E
14	H	R	R	A	T	Q	E
15	H	R	R	T	S	Q	E
16	H	H	R	A	T	Q	A
17	H	H	R	A	T	Q	E
18	H	H	H	A	T	Q	E

[0065] According to another aspect of the present invention there is provided a polynucleotide which encodes any human $P2X_7$ polypeptide combination of allelic variants defined herein.

[0066] The invention will now be illustrated but not limited by reference to the following Examples. All temperatures are in degrees Celsius.

[0067] In the Examples below, unless otherwise stated, the following methodology and materials have been applied.

[0068] AMPLITAQ™ ,available from Perkin-Elmer Cetus, is used as the source of thermostable DNA polymerase.

[0069] General molecular biology procedures can be followed from any of the methods described in "Molecular Cloning - A Laboratory Manual" Second Edition, Sambrook, Fritsch and Maniatis (Cold Spring Harbor Laboratory, 1989) or "Current Protocols in Molecular Biology", Volumes 1-3 , Edited by F M Asubel, R Brent & R E Kingston, published by John Wiley, 1998.

[0070] Electropherograms were obtained in a standard manner: data was collected by ABI377 data collection software and the wave form generated by ABI Prism sequencing analysis (2.1.2).

Example 1

Identification of Polymorphisms

1. Methods

DNA Preparation

[0071] DNA was prepared from frozen blood samples collected in EDTA following protocol I (Molecular Cloning: A Laboratory Manual, p392, Sambrook, Fritsch and Maniatis, 2nd Edition, Cold Spring Harbor Press, 1989) with the following modifications. The thawed blood was diluted in an equal volume of standard saline citrate instead of phosphate buffered saline to remove lysed red blood cells. Samples were extracted with phenol, then phenol/chloroform and then chloroform rather than with three phenol extractions. The DNA was dissolved in deionised water.

Template Preparation

[0072] Templates were prepared by PCR using the oligonucleotide primers and annealing temperatures set out below. The extension temperature was 72° and denaturation temperature 94°. Generally 50 ng of genomic DNA was used in each reaction and subjected to 35 cycles of PCR. Where described below. the primary fragment was diluted 1/100 and two microlitres were used as template for amplification of secondary fragments. PCR was performed in two stages (primary fragment then secondary fragment) to ensure specific amplification of the desired target sequence.

Polymorphisms in $P2X_7$

[0073]

Region	Size	Polymorphism	protein change	frequency
5'UTR		936 C→A 1012 T→C 1147 A→G 1343 G→A 1476 A→G		3/56 42/56 3/56 2/52 35/52
exon 1	146bp			
intron A	21.7kb			
exon 2	168bp	253 T→C	val76ala	2/54
intron B	1.1kb			
axon 3	68bp			
intron C	4.7kb			
exon 4	73bp			
intron D	1.5kb			
axon 5	95bp	488 G→A 489 C→T	silent his155tyr	2/54 17/38
intron E	2.8kb	4780 C→T 4845 C→T 4849 A→C		39/52 39/52 28/36
exon 6	80bp			
intron F	617bp	5021 T→C 5554 (GTTT)n=3,4 5579 G→C 5535 A→T		1/34 n=3, 14/40 26/40 1/44
exon 7	129bp	760 T→G	val245gly	1/40
intron G	1.3kb	5845 C→T 6911 T→C		2/40 33/50
exon 8	136bp	835 G→A 853 G→A	arg270his arg276his	16/52 1/54
intron H				
exon 9	91bp			
intron I	1.7kb			
exon 10	64bp			
intron J	84bp			

(continued)

Region	Size	Polymorphism	protein change	frequency
exon 11	149bp	1068 G→A	ala348thr	18/62
		1096 C→G	thr357ser	5/66
intron K				
exon 12	101bp	1315 C→G	pro430arg, splice site	4/66
intron L	3.8kb			
exon 13	497bp	1324 C→T	ala433val	1/54
		1405 A→G	gln460arg	3/54
		1448 C→T	silent	2/54
		1494 A→G	ser490gly	2/54
		1513 A→C	glu496ala	8/54
		1628 G→T	silent	2/52
		1772 G→A	silent	24/54

Positions in the 5' UTR refer to SEQ ID NO: 1.
Positions in exons refer to SEQ ID NO: 2.
Positions in introns refer to SEQ ID NO: 3.
Positions in protein refer to SEQ ID NO: 4.

[0074] Evidence for effects of some polymorphisms on transcription are as follows. C at position 1012 SEQ ID No 1 disrupts the TCAAT motif from an enhancer binding sequence reported in intron 1 of EGFR. A at position 1147 SEQID No 1 disrupts the reverse sequence of the TCCTGC motif which is also an enhancer binding sequence from intron 1 EGFR. (Maekawa T., Imamoto F., Merlino G. T., Pastan I., Ishii S.
Cooperative Function of Two Separate Enhancers of RT the Human Epidermal Growth
Factor Receptor Proto-oncogene J. BioL Chem. 264:5488-5494 (1989)).

Example 2

Haplotype analysis

a) The following allele frequencies were determined in a Swedish population.

[0075]

SEQ ID NO	Position	Frequency
1	1012	46/60
2	489	27/60
3	5579	39/60
2	835	16/58
2	853	3/60
2	1068	24/58
2	1096	6/58
2	1045	11/60
2	1513	10/60

b) Haplotype data.

[0076] Analysis of 15 Swedish families with at least one asthmatic child using primer extension (SNapShot™, Perkin

Elmer) genotyping and GeneHunter™ analysis demonstrated the following haplotypes:

	1012	489	5579	835	853	1068	1096	1405	1513	Frequency n/58
1	T	T	C	G	G	A	G	A	A	1
2	C	C	G	G	G	G	C	A	A	3
3	C	C	C	A	G	G	C	A	C	1
4	C	T	G	G	G	A	C	G	A	5
5	C	C	G	G	G	A	G	A	A	1
6	C	C	C	A	G	G	C	A	A	8
7	T	T	G	G	G	A	C	G	A	1
8	C	T	C	G	G	G	C	A	A	3
9	C	C	C	G	G	A	C	A	A	3
10	C	T	G	G	G	G	C	A	C	2
11	T	C	G	G	G	A	C	A	A	2
12	C	T	C	G	G	G	C	A	C	3
13	T	C	C	G	G	A	C	A	A	4
14	T	C	C	G	G	G	C	A	C	1
15	C	T	C	G	G	A	C	A	A	2
16	T	T	C	G	G	A	C	G	A	1
17	C	C	G	G	G	A	C	G	A	2
18	T	C	G	A	A	G	C	A	A	2
19	C	C	C	G	G	G	G	A	A	1
20	T	C	C	G	G	G	G	A	A	1
21	C	T	C	A	G	G	C	A	A	4
22	C	C	C	G	G	G	C	A	C	3
23	C	T	G	G	A	A	G	G	A	1
24	T	T	G	G	G	A	G	G	A	1
25	C	T	C	G	G	G	G	A	A	1
26	C	C	C	G	G	G	C	A	A	1

This results in the following proteins:

position SEQ ID NO 4	155	270	276	348	357	460	496	Frequency N/58
amino acid	Y	R	R	T	S	Q	E	1
	Y	R	R	T	T	R	E	7
	Y	R	R	T	T	Q	E	2
	Y	R	R	T	S	R	E	1
	Y	R	R	A	T	Q	A	5
	Y	R	R	A	T	Q	E	3
	Y	R	R	A	S	Q	E	1
	Y	R	H	T	S	R	E	1

(continued)

position SEQ ID NO 4	155	270	276	348	357	460	496	Frequency N/58
	Y	H	R	A	T	Q	E	4
	H	R	R	T	T	Q	E	9
	H	R	R	T	T	R	E	2
	H	R	R	A	T	Q	A	4
	H	R	R	A	S	Q	E	3
	H	R	R	A	T	Q	E	3
	H	R	R	T	S	Q	E	1
	H	H	R	A	T	Q	A	1
	H	H	R	A	T	Q	E	8
	H	H	H	A	T	Q	E	2

c) Analysis

[0077] Ben J. Gu, Weiyi Zhang, Rebecca A. Worthington, Ronald Sluyter, Phuong Dao-Ung, Steven Petrou, Julian A. Barden, and James S. Wiley, J. BioL Chem. (2001) 276: 11135-11142 reported that Ala at 496 (C at 1513) leads to loss of function in P2X7. Only one polymorphism was reported since they only analysed the final exon for SNPs

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Claims

1. A method for the diagnosis of a polymorphism in $P2X_7$ in a human, which method comprises determining the sequence of the human at one or more of the following positions:

   positions 936, 1012, 1147, 1343 and 1476 in the 5'UTR region of the $P2X_7$ gene as defined by the position in SEQ ID NO: 1;
   positions 253, 488, 489, 760, 835, 853, 1068, 1096, 1315, 1324, 1405, 1448, 1494, 1513, 1628 and 1772 in the coding region of the $P2X_7$ gene as defined by the position in SEQ ID NO: 2; and
   positions 4780, 4845, 4849, 5021, 5554, 5579, 5535, 5845 and 6911 in the intron region of the $P2X_7$ gene as defined by the position in SEQ ID NO: 3;
   positions 76 ,155, 245, 270, 276, 348, 357, 430, 433, 460, 490 and 496 in the $P2X_7$ polypeptide as defined by the position in SEQ ID NO: 4;

   and determining the status of the human by reference to polymorphism in $P2X_7$.

2. Use of a diagnostic method as defined in claim 1 to assess the pharmacogenetics of a drug acting at $P2X_7$.

3. A polynucleotide comprising at least 20 bases of the human $P2X_7$ gene and comprising an allelic variant selected from any one of the following:

Region	Variant SEQ ID NO: 1
5'UTR	936 A 1012 C 1147 G 1343 A 1476 G

Region	Variant SEQ ID NO: 2
exon 2	253 C
exon 5	488 A 489 T
exon 7	760 G
exon 8	835 A 853 A
exon 11	1068 A 1096 G
exon 12	1315 G
exon 13	1324 T 1405 G 1448 T 1494 G 1513 C 1628 T 1772 A

Region	Variant SEQ ID NO: 3
intron E	4780 T 4845 T 4849 C
intron F	5021 C 5554 $(GTTT)_n$,n=4 5579 C 5535 T
intron G	5845 T 6911 C

4. A nucleotide primer which can detect a polymorphism as defined in claim 1.

5. An allele specific primer capable of detecting a $P2X_7$ gene polymorphism as defined in claim 1.

6. An allele-specific oligonucleotide probe capable of detecting a $P2X_7$ gene polymorphism as defined in claim 1.

7. Use of a $P2X_7$ gene polymorphism as defined in claim 1 as a genetic marker in a linkage study.

8. A method of treating a human in need of treatment with a drug acting at $P2X_7$ in which the method comprises:

i) diagnosis of a polymorphism in $P2X_7$ in the human, which diagnosis preferably comprises determining the

sequence at one or more of the following positions:

positions 936, 1012, 1147, 1343 and 1476 in the 5'UTR region of the $P2X_7$ gene as defined by the position in SEQ ID NO: 1;
positions 253, 488, 489, 760, 835, 853, 1068, 1096, 1315, 1324, 1405, 1448, 1494, 1513, 1628 and 1772 in the coding region of the $P2X_7$ gene as defined by the position in SEQ ID NO: 2; and
positions 4780, 4845, 4849, 5021, 5554, 5579, 5535, 5845 and 6911 in the intron region of the $P2X_7$ gene as defined by the position in SEQ ID NO: 3; and
positions 76 ,155, 245, 270, 276, 348, 357, 430, 433, 460, 490 and 496 in the $P2X_7$ polypeptide as defined by the position in SEQ ID NO: 4;
and determining the status of the human by reference to polymorphism in $P2X_7$; and

ii) administering an effective amount of the drug.

9. An allelic variant of human $P2X_7$ polypeptide comprising at least one of the following:

a alanine at position 76 of SEQ ID NO 4;
a tyrosine at position 155 of SEQ ID NO 4;
a glycine at position 245 of SEQ ID NO 4;
a histidine at position 270 of SEQ ID NO 4;
a histidine at position 276 of SEQ ID NO 4;
a threonine at position 348 of SEQ ID NO 4;
a serine at position 357 of SEQ ID NO 4;
a arginine at position 430 of SEQ ID NO 4;
a valine at position 433 of SEQ ID NO 4;
a arginine at position 460 of SEQ ID NO 4;
a glycine at position 490 of SEQ ID NO 4; and
a glutamic acid at position 496 of SEQ ID NO 4;

or a fragment thereof comprising at least 10 amino acids provided that the fragment comprises at least one allelic variant.

10. An antibody specific for an allelic variant of human $P2X_7$ polypeptide as defined in claim 9.

11. A polynucleotide comprising any one of the following twenty six $P2X_7$ haplotypes:

	1012	489	5579	835	853	1068	1096	1405	1513
	SEQ ID 1	SEQ ID 2	SEQ ID 3	SEQ ID 2	SEQ ID 2	SEQ ID 2	SEQ ID 2	SEQ ID 2	SEQ ID 2
1	T	T	C	G	G	A	G	A	A
2	C	C	G	G	G	G	C	A	A
3	C	C	C	A	G	G	C	A	C
4	C	T	G	G	G	A	C	G	A
5	C	C	G	G	G	A	G	A	A
6	C	C	C	A	G	G	C	A	A
7	T	T	G	G	G	A	C	G	A
8	C	T	C	G	G	G	C	A	A
9	C	C	C	G	G	A	C	A	A
10	C	T	G	G	G	G	C	A	C
11	T	C	G	G	G	A	C	A	A
12	C	T	C	G	G	G	C	A	C

(continued)

	1012	489	5579	835	853	1068	1096	1405	1513
	SEQ ID 1	SEQ ID 2	SEQ ID 3	SEQ ID 2	SEQ ID 2	SEQ ID 2	SEQ ID 2	SEQ ID 2	SEQ ID 2
13	T	C	C	G	G	A	C	A	A
14	T	C	C	G	G	G	C	A	C
15	C	T	C	G	G	A	C	A	A
16	T	T	C	G	G	A	C	G	A
17	C	C	G	G	G	A	C	G	A
18	T	C	G	A	A	G	C	A	A
19	C	C	C	G	G	G	G	A	A
20	T	C	C	G	G	G	G	A	A
21	C	T	C	A	G	G	C	A	A
22	C	C	C	G	G	G	C	A	C
23	C	T	G	G	A	A	G	G	A
24	T	T	G	G	G	A	G	G	A
25	C	T	C	G	G	G	G	A	A
26	C	C	C	G	G	G	C	A	A

12. A human $P2X_7$ polypeptide comprising one of the following eighteen combinations of alleleic variant determined amino acids based on positions identified in SEQ ID NO: 4:

	155	**270**	**276**	**348**	**357**	**460**	**496**
1	Y	R	R	T	S	Q	E
2	Y R		R	T	T	R	E
3	Y	R	R	T	T	Q	E
4	Y	R	R	T	S	R	E
5	Y	R	R	A	T	Q	A
6	Y	R	R	A	T	Q	E
7	Y	R	R	A	S	Q	E
8	Y	R	H	T	S	R	E
9	Y	H	R	A	T	Q	E
10	H	R	R	T	T	Q	E
11	H	R	R	T	T	R	E
12	H	R	R	A	T	Q	A
13	H	R	R	A	S	Q	E
14	H	R	R	A	T	Q	E
15	H	R	R	T	S	Q	E
16	H	H	R	A	T	Q	A
17	H	H	R	A	T	Q	E
18	H	H	H	A	T	Q	E

13. A polynucleotide which encodes any human $P2X_7$ polypeptide as defined in claim 12.

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(11) EP 1 199 372 A3

(12) EUROPEAN PATENT APPLICATION

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(54) Polymorphisms in the human P2X7 gene

(57) This invention relates to polymorphisms in the human $P2X_7$ gene and corresponding novel allelic polypeptides encoded thereby. The invention also relates to methods and materials for analysing allelic variation in the $P2X_7$ gene, and to the use of $P2X_7$ polymorphism in treatment of diseases with $P2X_7$ drugs.

EP 1 199 372 A3

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PARTIAL EUROPEAN SEARCH REPORT

which under Rule 45 of the European Patent Convention shall be considered, for the purposes of subsequent proceedings, as the European search report

Application Number EP 01 30 8837

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
X	WO 97 40462 A (SPECTRA BIOMEDICAL INC) 30 October 1997 (1997-10-30) * abstract; claim 1 * ---	1-7	C12Q1/68C07K16/28 C12Q1/68
X	HALUSHKA M K ET AL: "Patterns of single-nucleotide polymorphisms in candidate genes for blood-pressure homeostasis." NATURE GENETICS. UNITED STATES JUL 1999, vol. 22, no. 3, July 1999 (1999-07), pages 239-247, XP000985696 ISSN: 1061-4036 * page 239 * ---	1-7	
X	BROOKES A J: "The essence of SNPs." GENE. NETHERLANDS 8 JUL 1999, vol. 234, no. 2, 8 July 1999 (1999-07-08), pages 177-186, XP004173090 ISSN: 0378-1119 * the whole document * --- -/--	1-7	

TECHNICAL FIELDS SEARCHED (Int.Cl.7)

C12Q

INCOMPLETE SEARCH

The Search Division considers that the present application, or one or more of its claims, does/do not comply with the EPC to such an extent that a meaningful search into the state of the art cannot be carried out, or can only be carried out partially, for these claims.

Claims searched completely :

Claims searched incompletely :

Claims not searched :

Reason for the limitation of the search:

see sheet C

Place of search	Date of completion of the search	Examiner
MUNICH	12 March 2004	Costa Roldán, N

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

EPO FORM 1503 03.82 (P04C07)

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INCOMPLETE SEARCH SHEET C

Application Number
EP 01 30 8837

Claim(s) searched completely:
1-7,9-13

Claim(s) searched incompletely:
8

Reason for the limitation of the search (non-patentable invention(s)):

Article 52 (4) EPC - Method for treatment of the human or animal body by therapy

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Further limitation of the search

Claim(s) searched completely:
1-3,5-7,9-13

Claim(s) searched incompletely:
4

Claim(s) not searched:
8

Reason for the limitation of the search:

Claim 4 is directed to a primer, which is defined only in terms of a result to be achieved, i.e to detect a polymorphism. Thus, such a primer could derive from practically any genomic portion upstream of the polymorphism. Therefore, said claim is unclear (Art. 84 EPC) and has been searched only in so far as it relates to primers of 17 to 50 nucleotides in length which are complementary or identical to portions of the P2X7 gene.

Claim 8 was not searched because it is directed to a method of treatment using a drug acting at P2X7 gene, but the application does not identify any such drugs therefore claim 8 lacks clarity (Art. 84EPC) to the extent that no search is possible.

European Patent Office

PARTIAL EUROPEAN SEARCH REPORT

Application Number
EP 01 30 8837

DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	
X	US 6 133 434 A (BUELL GARY NUTTER ET AL) 17 October 2000 (2000-10-17)	4,9	
A	* column 35 - column 38; claim 7 *	1-3,5-7, 10-13	
	* column 37 - column 40; claim 1 *		
D,X	BUELL G N ET AL: "Gene structure and chromosomal localization of the human P2X7 receptor." RECEPTORS & CHANNELS. SWITZERLAND 1998, vol. 5, no. 6, 1998, pages 347-354, XP009021403 ISSN: 1060-6823	4	
A	* the whole document *	1-3,5-7, 9-13	TECHNICAL FIELDS SEARCHED (Int.Cl.7)
D,A	WO 99 29660 A (ASTRA PHARMA PROD ;CLADINGBOEL DAVID (GB); MORTIMORE MICHAEL (GB);) 17 June 1999 (1999-06-17) * abstract *	1-7,9-13	
A	LYNCH K J ET AL: "MOLECULAR AND FUNCTIONAL CHARACTERIZATION OF HUMAN P2X2 RECEPTORS" MOLECULAR PHARMACOLOGY, BALTIMORE, MD, US, vol. 56, no. 6, December 1999 (1999-12), pages 1171-1181, XP000876836 ISSN: 0026-895X * page 1171 *	1-7,9-13	
	-/—		

EPO FORM 1503 03.82 (P04C10)

European Patent Office

PARTIAL EUROPEAN SEARCH REPORT

Application Number
EP 01 30 8837

DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	
P,X	WILEY J S ET AL: "GENETIC POLYMORPHISMS OF THE HUMAN P2X7 RECEPTOR AND RELATIONSHIP TO FUNCTION" DRUG DEVELOPMENT RESEARCH, NEW YORK, NY, US, vol. 53, no. 2/3, June 2001 (2001-06), pages 72-76, XP001119468 ISSN: 0272-4391 * page 72; table 1 *	1-7,9-13	
P,X	GU BEN J ET AL: "A Glu-496 to Ala polymorphism leads to loss of function of the human P2X7 receptor" JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 276, no. 14, 6 April 2001 (2001-04-06), pages 11135-11142, XP002263729 ISSN: 0021-9258 * the whole document *	1-7,9-13	TECHNICAL FIELDS SEARCHED (Int.Cl.7)

EPO FORM 1503 03.82 (P04C10)

European Patent Office

Application Number
EP 01 30 8837

CLAIMS INCURRING FEES

The present European patent application comprised at the time of filing more than ten claims.

☐ Only part of the claims have been paid within the prescribed time limit. The present European search report has been drawn up for the first ten claims and for those claims for which claims fees have been paid, namely claim(s):

☐ No claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for the first ten claims.

LACK OF UNITY OF INVENTION

The Search Division considers that the present European patent application does not comply with the requirements of unity of invention and relates to several inventions or groups of inventions, namely:

see sheet B

☐ All further search fees have been paid within the fixed time limit. The present European search report has been drawn up for all claims.

☐ As all searchable claims could be searched without effort justifying an additional fee, the Search Division did not invite payment of any additional fee.

☒ Only part of the further search fees have been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the inventions in respect of which search fees have been paid, namely claims:

1-13 (all partially), inventions 1 and 28

☐ None of the further search fees have been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the invention first mentioned in the claims, namely claims:

European Patent Office

LACK OF UNITY OF INVENTION
SHEET B

Application Number
EP 01 30 8837

The Search Division considers that the present European patent application does not comply with the requirements of unity of invention and relates to several inventions or groups of inventions, namely:

Inventions 1: claims 1-8 (partially)

Invention 1

A polynucleotide comprising at least 20 bases of the human P2X7 gene comprising the allelic variant 936 A, the use of said polymorphism as a genetic marker in a linkage study, probes and primers for the detection of said polymorphism; and a method for the diagnosis of said polymorphism for determining the status of a human.

Invention 2-16: claims 1-8 (partially)

Inventions 2 to 16

ibid for SNPs at nucleotide positions 1147, 1343, 1476, 488, 1448, 1628, 1772, 4780, 4845, 4849, 5021, 5554, 5535, 5845, 6911.

Inventions 17-21: claims 1-10 (partially)

Invention 17

A polynucleotide comprising at least 20 bases of the human P2X7 gene comprising the allelic variant 253 C, an allelic variant of human P2X7 polypeptide comprising an Alanine at position 76 of SEQ ID NO:4, the use of said polymorphism as a genetic marker in a linkage study, probes and primers for the detection of said polymorphism; and a method for the diagnosis of said polymorphism for determining the status of a human.

Inventions 18 to 21

ibid for SNPs at nucleotide positions:

760 (the allelic variant of human P2X7 polypeptide comprising a Glycine at position 245 of SEQ ID NO:4),

1315 (the allelic variant of human P2X7 polypeptide comprising an Arginine at position 430 of SEQ ID NO:4),

1324 (the allelic variant of human P2X7 polypeptide comprising a Valine at position 433 of SEQ ID NO:4),

1494 (the allelic variant of human P2X7 polypeptide comprising a Glycine at position 490 of SEQ ID NO:4)

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LACK OF UNITY OF INVENTION SHEET B

Application Number
EP 01 30 8837

The Search Division considers that the present European patent application does not comply with the requirements of unity of invention and relates to several inventions or groups of inventions, namely:

Inventions 22-28: claims 1-13 (partially)

Invention 22

A polynucleotide comprising at least 20 bases of the human P2X7 gene comprising the allelic variant 489 T; an allelic variant of human P2X7 polypeptide comprising an Tyrosine at position 155 of SEQ ID NO:4; the polynucleotide which encodes said polypeptides; the use of said polymorphism as a genetic marker in a linkage study, probes and primers for the detection of said polymorphism; and a method for the diagnosis of said polymorphism for determining the status of a human.

Invention 23 to 28

ibid for SNPs at nucleotide positions:

835 (the allelic variant comprising a Histidine at position 270 of SEQ ID NO:4),

853 (the allelic variant comprising a Histidine at position 276 of SEQ ID NO:4)

1068 (the allelic variant comprising a Threonine at position 348 of SEQ ID NO:4)

1096 (the allelic variant comprising a Serine at position 357 of SEQ ID NO:4)

1405 (the allelic variant comprising Arginine at position 460 of SEQ ID NO:4)

1513 (the allelic variant comprising a Glutamic acid at position 496 of SEQ ID NO:4)

Inventions 29-30 : claims 1-8 and 11 (partially)

Invention 29

A polynucleotide comprising at least 20 bases of the human P2X7 gene comprising the allelic variant 1012 C; the use of said polymorphism as a genetic marker in a linkage study, probes and primers for the detection of said polymorphism; and a method for the diagnosis of said polymorphism for determining the status of a human.

European Patent Office

LACK OF UNITY OF INVENTION
SHEET B

Application Number
EP 01 30 8837

The Search Division considers that the present European patent application does not comply with the requirements of unity of invention and relates to several inventions or groups of inventions, namely:

Invention 30

A polynucleotide comprising at least 20 bases of the human P2X7 gene comprising the allelic variant 5579 C; the use of said polymorphism as a genetic marker in a linkage study, probes and primers for the detection of said polymorphism; and a method for the diagnosis of said polymorphism for determining the status of a human.

ANNEX TO THE EUROPEAN SEARCH REPORT ON EUROPEAN PATENT APPLICATION NO. EP 01 30 8837

This annex lists the patent family membersrelating to the patent documents cited in the above-mentioned European search report. The members are as contained in the European Patent Office EDP file on
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12-03-2004

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